Vascular diseases await translation of blood vessels engineered from stem cells

Engineering blood vessels from human induced pluripotent stem cells: Implications for vascular disease models and clinical translation

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Abstract

The discovery of human induced pluripotent stem cells (hiPSCs) opened new avenues for research and potential clinical applications for tissue regeneration and engineering. In particular, hiPSCs may offer a long sought solution for obtaining large numbers of autologous cells sufficient for tissue engineering. For vascular tissue engineering, several methods of generating endothelial cells or perivascular cells from hiPSCs *in vitro* have been reported. We review the current developments in the generation of vascular progenitor cells from hiPSCs and their functional capacity *in vivo*, and discuss the opportunities and challenges ahead for clinical translation and modeling vascular diseases using hiPSC-derived vascular cells with a focus on diabetes.

One sentence summary:

Successful generation of hiPSC-derived vascular cells *in vitro* and functional blood vessels *in vivo* offers a hope for clinical translation, yet important hurdles remain.

Introduction

Two types of cells are required to form a stable blood vessel: endothelial cells (ECs) and the perivascular cells (PVCs: pericytes or vascular smooth muscle cells)[1-6]. Stem cells have the potential to provide a limitless supply of proliferative progenitor cells that could be differentiated into vascular cells *in vivo* to form a mature and durable network of blood vessels[7,8]. However, adult stem cells have only limited proliferative potential, diminished differentiation capacity and increased senescence[9]. Human induced pluripotent stem cells (hiPSCs) offer a promising alternative[10-12]. Indeed, retroviral transduction of only 4 transcription factors is able to 'reprogram' terminally differentiated cells of adult origin into pluripotent – an embryonic stem cell-like – state. The hiPSCs can, in principle, provide a rich source of non-immunogenic allogeneic stem or progenitor cells, capable of differentiation and organization into functional tissue in a biologically relevant microenvironment. Additionally, the use of hiPSC derivatives in humans would be free of ethical issues that arise with another pluripotent cell source – the human embryonic stem cells (hESCs)[13,14].

Creation of stable and functional blood vessels *in vivo* from hiPSCs has tremendous potential for repairing diseased state of vasculature, tissue engineering and organ transplantation. Another vital application of hiPSC-derived blood vessels is the ability to examine the mechanisms of and test novel strategies to correct various vascular pathologies "in a dish"– such as diabetic vascular complications and abnormal vascular development. However, the challenge for any of these applications remains the ability to derive adequate numbers of healthy progeny from hiPSC-derived vascular progenitor cells that can form functional vessels *in vivo* and thus, could be used for clinical translation or disease modeling. We will give a brief historical overview of vascular tissue engineering followed by the discussion about new developments in

the generation of vascular progenitor cells from hiPSCs, their functional capacity *in vivo*, and opportunities and challenges for clinical translation in vascular disease such as diabetes.

Vascular tissue engineering: The pre-hiPSC era

The formation of blood vessels occurs mainly by two processes: 1) angiogenesis – the formation of new vessels from existing blood vessels, and 2) vasculogenesis – *de novo* blood vessel formation[2,15,16]. Vascular tissue engineering involves an intricately orchestrated series of cellular and molecular events engaging vascular progenitor cells and non-vascular cells in a specialized microenvironment[2,3,17]. The applications of vascular tissue engineering include generation of vascular grafts[18], and revascularization in chronic limb ischemia[19]. Significant developments in vascular tissue engineering include exploration of different sources of vascular cells, biologically relevant animal models of disease, sophisticated techniques for *in vivo* visualization of engineered blood vessels, and mechanistic insights[4,20-23].

The growth factors and signaling pathways in vascular cells that control vasculogenesis and angiogenesis include vascular endothelial growth factor (VEGF) family, basic fibroblast growth factor (b-FGF), platelet derived growth factor (PDGF)-BB, Hedgehog family, Notch, semaphorins, Tie-2 and angiopoietins[2,24-29]. The controlled release of angiogenic growth factors in combination of extracellular matrices, biochemical and mechanical cues favor generation of blood vessels. The sequential delivery of VEGF and PDGF within scaffolds facilitated the formation of mature, remodeled blood vessels that alleviated hind limb ischemia in immunodeficient mice[30]. Another approach consisted of implantation of ECs, either alone or with perivascular cells, within appropriate scaffolds. Bovine aortic ECs– when co-implanted with 10T½ mouse embryonic mesenchymal stem/progenitor cells – caused the migration of 10T½ cells towards the ECs, and upon contact, led to mesenchymal mural phenotype of 10T½

cells *in vitro*[31]. Elegant mechanistic work demonstrated that the PVC facilitated stability of blood vessels via cross talk with ECs, a process that seems to be mediated PDGF-B and transforming growth factor-β (TGF-β)[31,32]. However, the exact mechanisms that facilitate stabilization of blood vessels and integration in host circulation *in vivo* still remain elusive.

Using pre-cellularized matrices for vascular engineering, we have successfully generated durable vascular networks *in vivo* using human umbilical vein ECs (HUVECs) along with 10T½ cells[33]. Our laboratory also established techniques for utilizing chronic "transparent" windows in animal models that allow visualization of vessel formation *in vivo* using a non-invasive imaging technique – laser scanning multiphoton microscopy – to examine the function of tissue engineered blood vessels[4,20-23,33-37]. Using this approach, we demonstrated that microvascular networks formed by co-implanting HUVECs with 10T½ cells remained durable and functional for nearly one year[33]. Furthermore, using this approach we revealed the mechanism of anastomosis of engineered vessels to host vasculature *in vivo*. Interestingly, we found that implanted ECs wrap around nearby host vessels, and subsequently cause reorganization of basement membrane and PVCs that leads to displacement of the host endothelium – a process termed "wrapping and tapping"[23].

In an attempt to make this approach more translatable, we have further developed approaches using peripheral blood endothelial progenitor cells (EPCs) instead of HUVECs. Adult peripheral blood EPCs-derived blood vessels regressed within 3 weeks, whereas umbilical cord blood EPCs formed a functional vasculature, which lasted for more than 4 months – indicating that "stemness" plays a key role. This could be attributed to a higher proliferative capacity of cord blood derived EPCs when compared to peripheral blood EPCs as demonstrated by a higher proliferative Ki-67 index. Furthermore, cord blood-derived EPCs were more resistant

to stress induced apoptosis as compared to peripheral blood-derived EPCs, indicating a greater survival advantage of cord blood EPCs[20]. Ingram et al have demonstrated that cord blood-derived EPCs have a higher level of telomerase activity as compared to peripheral blood-derived EPCs[38]. We also replaced the murine 10T½ cells with bone marrow-derived human mesenchymal stem cells (hMSCs), while others have used adipose tissue derived stem cells[4,20,21,23,35-37,39,40]. The MSCs could differentiate into PVCs with a pericyte-like phenotype that stabilized vessels *in vivo*[34]. Finally, derivation of vascular progenitor cells from hESCs[35,41-51] generated tremendous excitement as a limitless source of cells that might be used for vascular tissue engineering[35,44-46,51,52]. To this end, we created functional blood vessels *in vivo* in mice by co-implanting hESC-ECs with 10T½ cells. These vascular networks lasted for at least 150 days[35]. Although hESCs remain the gold standard of pluripotency and our data on hESC-ECs provided a proof of the principle for the stem cell-derived tissue engineered blood vessels, the use of hESCs in the clinic remains controversial.

Despite the above-mentioned advances in vascular tissue engineering, important challenges remain. Cell therapy-using EPCs has not been successful in generating robust vascular networks[53]. Therapeutic strategies using pro-angiogenic cells for peripheral vascular disease[54] and ischemic heart disease[55] have led to only modest functional improvement. These challenges in the field of vascular tissue engineering have so far precluded successful application in the clinic. It is known that vascular cells that are exposed to a hypoxic environment are vulnerable to cell death *in vivo*[56]. Given the heterogeneity of ECs, functionally viable engineered blood vessels may need a full complement of or specific ECs to form blood vessels including capillaries, arteries and venules[57]. One-size may not fit all also for perivascular cells, which vary depending on anatomic location, for instance PVC coverage is very tight in the brain vessels but

scant in vessels in the skeletal muscle[1]. Besides engineering the cellular components, it is equally relevant to pay attention to the biological composition of the scaffold that forms the microenvironment for EC/PVC interaction and stability. Inherent EC dysfunction and senescence of vascular cells with advanced aging is also likely to be also important[9,58,59].

The human induced pluripotent stem cell (hiPSC) era

After Yamanaka's groundbreaking method of reprogramming adult human fibroblasts using retroviral transduction of Oct-4 (octamer-binding transcription factor-4 also known as POU5F1), Sox-2 (SRY-related high-mobility-group (HMG)-box protein-2), Klf-4 (Kruppel-like factor 4) and c-Myc (cellular homolog of the myelocytomatosis viral oncogene)[11], numerous other non-integrating approaches have been reported. These included viral[60-63], transgene free[64-67], and non-viral, including mRNA[68,69] and small molecules[70-72]. These potentially safer methods of reprogramming offer the hope of clinical translation of hiPSC- derivatives, particularly those that offer zero-footprint technology. The roadblock to clinical translation included poor reprogramming efficiencies and a lack of full mechanistic understanding of the barriers to reprogramming[14]. It has recently been demonstrated that totipotent mouse iPSCs generated *in vivo* were closer to embryonic stem cells than *in vitro* generated hiPSCs[73]. The selected inhibition of a single factor, MBD3 (epigenetic repressor) radically amplified the reversion efficiency of primed mouse pluripotent epiblast cells to ground state pluripotency. Furthermore, more than 98% of mouse embryonic fibroblasts were reprogrammed in a week's time, when the traditional 4 Yamanaka factors were combined with the genetic depletion of MBD3[74]. These reports have resulted in an enhanced understanding of reprogramming mechanisms in mice and hiPSCs.

An outstanding issue for the field is whether the cell of origin of hiPSC (source) or

efficiency of reprogramming greatly influences differentiation potential of hiPSC cells[75]. The "memory" of hiPSC cells (expression of genes reflecting origin of cells) may diminish over passages in culture. Ohi et al. reported somatic memory of gene expression in all hiPSC lines examined that correlated with retention of the demethylated status of genes normally methylated in ESCs[76]. This gene expression profile was noted even in hiPSC cells that were derived using non-viral, non-integrating techniques. Of greater concern is that the genes expressed have been associated with cancer, and thus a careful examination of the genome and epigenome of hiPSC-derived cell lines is warranted prior to the clinical translation. Interestingly, Ohi et al. found that hepatocyte-derived hiPSC cells did not show a greater tendency to generate endoderm when compared with fibroblast-derived hiPSC cells[76]. Kim et al. demonstrated that blood-derived hiPSCs were less efficient in differentiating into keratinocytes than hESC cells suggesting that blood derived hiPSC cells may not be efficient in skin regeneration[77]. More recently, a comparison in differentiation properties of genetically matched hiPSC clones from bone marrow and dermal fibroblasts from the same patient did not reveal any significant differences in global gene expression profiles[78].

The above concerns notwithstanding, the first hiPSC-based clinical trial using retinal pigment epithelial cells recently opened[79,80]. Companies such as Advanced Cell Technology have approached the Food and Drug Administration (FDA) in the United States for testing platelets derived from hiPSCs on humans[80]. A proposed trial using genetically corrected keratinocytes to treat Epidermolysis Bullosa by Stanford researchers is currently under review by the FDA (http://www.cirm.ca.gov/our-progress/awards/ips-cell-based-treatment-dominant-dystrophic-epidermolysis-bullosa). In July 2013, the first human clinical study using hiPSC derived retinal pigment epithelial cells was approved for injection into the eyes of six patients diagnosed with

age-related macular degeneration, a condition that leads to blindness. As of September 1st, 2014, this trial was underway. This was first study in which genetically matched skin cells from age-related macular degeneration patients that have been reprogrammed to hiPSCs and differentiated to retinal cells were implanted back into the patients[79-81]. The expectation of such a study is that the hiPSC-retinal cells will prevent ongoing destruction of photoreceptors. However, this approach is unlikely to repair vision that has already been lost. If successful, the landscape for hiPSC-derivatives for human translation will widen, dramatically[79]. The "proof of concept" for retinal sheet transplantation therapy for advanced retinal degenerative diseases has recently been demonstrated in mice [82].

The hope for hiPSC-derived tissue in regenerative medicine has been replacement of tissues or organs that would otherwise require transplantation. (Stem Cell Research: Trends and perspectives on the evolving international landscape: "http://www.elsevier.com/online-tools/research-intelligence/research-initiatives/stem-cell-research"). Indeed, hiPSC-derived hepatocytes produced a functional vascularized liver in mice[83]. hiPSC-derived hepatocytes, HUVECs and MSCs were used to engineer the liver organoids. It appears that a combination of cell types, and their cross talk is required for generating a functioning organoid such as the liver that can recapitulate physiological function *in vivo.* Significantly, the HUVECs were able to form blood vessels in the liver organoids as co-implanted MSCs were likely PVCs and stabilized the nascent vasculature[84]. Cerebral organoids or mini-brains derived from hiPSCs were created in a dish, and grew to their maximum size in 2 months[85]. The lack of vasculature, oxygen and nutrients to the core of the cerebral organoids may have prevented further development and growth.

Human induced pluripotent stem cells as sources of vascular endothelial cells

ECs have been derived from hiPSCs (hiPSC-ECs) from a variety of types of cells: including neonatal or adult skin fibroblasts[39,86-104], umbilical cord blood[102] and HUVECs[105]. Bone marrow derived CD34+ progenitor cells[106,107], amniotic fluid-derived cells[108], neonatal lung fibroblasts[106], dental pulp-derived cells[94,109], MSCs[104,110,111] and lipoaspirates[63] have also been converted to hiPSCs and subsequently differentiated into ECs. The diverse methods used to reprogram ECs from hiPSCs include lentiviral[10,37,86,89,90,94,97,103,104,107,110,112-114,115] retroviral[39,43,88,89,91-96,98,100,105,108,60,85,102,106,110,116,117], plasmid[37,106,107] modified RNA technology[86,102,110], 2-*D* or 3-*D* culture conditions, different combinations of transcription factors and feeder vs. feeder-free conditions. Derivation of hiPSC-ECs has also utilized a variety of selection markers (CD34, CD31, CD144/VE-Cadherin, CD146, Tie-2, FLT1, KDR, vWF) using flow cytometry of magnetic bead sorting as well as the addition of cytokines or pharmacologic agents in the culture media (BMP-4, VEGF-A, bFGF, Activin A, SCF, Flt3, Flt3L, IL-3, IL-6 or a MEK inhibitor) to promote EC differentiation (**Supplemental Tables S1** and **S2**).

Studies have found that the gene expression of hESC-ECs and hiPSC-ECs is largely similar[75]. A high level of concordance of gene expression was also noted between ECs derived from the same hiPSC line which was confirmed in multiple experiments and using different protocols for hiPSC-EC differentiation[75]. Other studies of hiPSC-ECs from different cell lines and using multiple differentiation protocols have reported variability between *in vitro* and *in vivo* functionality of hiPSC-EC networks[37]. Transient reprogramming techniques have also been shown to be sufficient to generate bona fide ECs[112].

A full complement of ECs can be derived from hiPSCs suggesting that hiPSC-ECs are functionally diverse and presumably similar to human ECs[57,96,102]. Rufaihah et al. have demonstrated that varying the exposure to soluble growth factors in differentiation protocols for

hiPSC leads to cell differentiation into arterial, venous or lymphatic ECs[96]. Culturing in high VEGF concentration media led to an arterial-EC phenotype, low VEGF levels promoted venous specification, and a combination of high VEGF-C and Angiopoietin-1 levels favored lymphatic differentiation. hiPSC-ECs formed the most robust capillary networks *in vivo* in subcutaneous Matrigel plugs implanted in Severe Combined Immunodeficiency Deficient (SCID) mice[96]. These results suggest that the hiPSC-arterial ECs may be beneficial for therapeutic angiogenesis in hind limb ischemia, whereas hiPSC-lymphatic ECs may be more advantageous for treatment of lymphedema. Indeed, immature EPCs show plasticity between arterial and venous phenotypes[116]. An arterial phenotype of ECs is believed to be induced by NOTCH signaling involving shear stress and facilitated by a high concentration of VEGF in media used for endothelial differentiation[75]. It has been suggested that identification of early vascular precursor markers such as KDR[117] or CD87[118] may provide clues into endothelial specification in development, and perhaps provide access to vasculogenic clones of hiPSC-ECs[37].

Human induced pluripotent stem cells as sources of mural/ perivascular cells

A prerequisite for vascular tissue engineering using hiPSC-ECs to become a reality is the availability of mural / perivascular cells (PVCs) such as pericytes and vascular smooth muscle cells (vSMCs) for stabilization and maturation of hiPSC-derived engineered blood vessels. Ideally these PVCs would be derived from the same autologous hiPSCs to facilitate clinical translation.

Pericytes share lineage derivation, morphological, tri-lineage differentiation into adipocytic, osteogenic and chondrogenic potential *in vivo*, and expression of common surface markers with MSCs[1]. Indeed MSCs or mesenchymal progenitor cells with a perivascular location *in vivo* are

termed pericytes or cells with pericyte-like morphology and function. Intermediate or transition states are also known to exist between the different mural cell types such as described in the derivation of functional vSMCs from hiPSCs via MSC intermediate cells[119].

Pericytes are considered the microvascular counterparts of vSMCs. The two mural cells are differentiated by morphology, and the expression of cytosolic and membrane bound markers[1,6]. For example, whereas pericytes are specifically perivascular in location and attached to the longitudinal axis of capillaries with finger-like extensions, vSMCs are positioned perpendicularly to the entire endothelial surface.

hiPSC-derived MSCs have been generated using neonatal or adult skin fibroblasts[104,119-132], human vascular smooth muscle[133], neonatal lung fibroblasts[99], bone marrow-derived CD34+ progenitor cells[106,108,128], hair keratinocytes[119,132] and brain[134] using retroviral[37,111,113,123,127,128,131,132,134-137], lentiviral[37,104,114,119,120,124,125,127,133,138], plasmid[99], [124,130] modified RNA technology[130], 2-*D* or 3-*D* culture conditions, and different combinations of transcription factors and feeder vs. feeder-free conditions_(**Supplemental Tables S1** and **S2)**[122,128]. The derivation of hiPSC-mural cells has utilized a variety of selection markers and addition of growth factors to culture conditions with varying efficiency. Flow cytometry for the following markers CD73, CD146, CD105, CD90, CD24, CD44, CD54, NG2, PDGF-β, HSP90, HLA-class I have been used to characterize hiPSC-derived MSCs. hiPSC- derived SMCs/vSMCs have been characterized using α-smooth muscle actin, calponin, SM22, TCF15, MYH11, ACTA2, CNN1, CSPG4. Additionally magnetic bead sorting and the addition of cytokines to culture media (BMP-4, DKK3, PD98059, Flt3, SCF, VEGF, IL-3, IL-6) have been employed to promote the differentiation of hiPSC-derived mural cells (**Supplemental Tables S1** and **S2)**.

Several reports showed hiPSC to MSC derivation and successful inoculation of these

hiPSC-MSCs in rodent models of ischemia[104,125,129]. However, based on the findings from the human bone marrow-derived MSCs, it is unclear how these MSCs participate in the revascularization, as the therapeutic properties of MSCs may also result from secretion of paracrine factors in addition to direct support[139,140]. Fibroblasts derived from hiPSCs and hESCs also support angiogenesis via paracrine mechanisms[77]. Dar et al. demonstrated that CD105+CD90+CD73+CD31– multipotent clonogenic mesodermal progenitor cells arising spontaneously from embryoid bodies (EBs) – termed 'multipotent clonogenic mesodermal precursors' – were positive for pericytic markers CD146, NG2 and PDGFR-β but were negative for α-smooth muscle actin. The cultured 'multipotent clonogenic mesodermal precursors' formed cartilage, bone, fat, and skeletal muscle cells and showed vasculogenic potential in Matrigel plugs when co-implanted with hESC-ECs or HUVECs. The EB-derived PVCs promoted *both* vascular and muscle regeneration in mice[132]. Besides the implications for using both ECs and pericytic derivatives from hiPSCs in vascular tissue engineering, this work also demonstrated that the hiPSC-EB model of PVC derivation may shed light on these elusive and often neglected perivascular-supporting cell. Another key finding was the generation of vascularized liver buds formed by the co-implantation of hiPSC-derived hepatocytes with non-hiPSC (HUVECs and MSCs). The development of the vascular network within the liver organoids within 2 days was attributed to the subset of MSCs behaving like pericytes, surrounding the HUVECs and stabilizing blood vessels, as has been demonstrated in the past[33,83].

Comparative studies suggest that hiPSC-derived SMCs are similar to the human SMC, sharing gene expression patterns, epigenetic modifications and *in vitro* functional properties. hiPSC-SMCs from human aortic vascular SMCs were re-differentiated back to SMCs, and were remarkably similar to the parenteral cells[133]. hiPSC-SMCs derived from three progenitor

subtypes (lateral plate mesoderm, paraxial mesoderm and neuroectoderm) suggest that organ specific SMCs exist and are an important resource to understand human vascular disease and development[131]. Quicker generation of hiPSC-perivascular cells has been enabled by using transient reprogramming techniques[99]. Furthermore the limited proliferative potential of human MSCs/SMCs seems to be offset by a step wise approach to generate functional hiPSC-SMCs through a highly proliferative MSC progenitor cell population[119]. Importantly, the hiPSC-SMCs can be directed to adopt either a synthetic or contractile phenotype[128].

Functional evaluation of hiPSC-derived vascular cells *in vivo*

Although several methods could be used to generate hiPSC-ECs, only a few studies have performed functional evaluation of hiPSC-ECs *in vivo,* with a follow up typically not extending beyond one month. hiPSC-ECs have promoted neovascularization *in vivo* in murine models of hind limb ischemia[91,92,94], myocardial infarction[63], and retinal ischemia/reperfusion injury[141,142]. hiPSC-ECs have also been shown to reduce collagen deposition in a mouse induced scleroderma model[95], and, in combination with hiPSCs derived SMCs, to enhance vascularization in a murine dermal wound model[104]. Remarkably, the functional response of hiPSC-ECs to proinflammatory stimuli has also been demonstrated *in vivo*. hiPSCs-ECs were able to respond to biomechanical cues thereby directing differentiation into cells of the circulatory system[110]. The hiPSC-ECs exhibited an activated phenotype in response to pro-inflammatory stimuli, and revealed anticoagulant properties. The ability to modulate hiPSC-ECs to develop athero-protective or athero-prone phenotypes is exciting and shows potential for drug screening to prevent plaques of atherosclerosis.

Ideally, the hiPSC-engineered blood vessel precursors to be used in the clinic should be Human Leukocyte Antigen (HLA)-matched. This could be achieved by generating both the ECs and

PVCs from the same individual. Indeed, our group and another one have demonstrated this possibility using different approaches[37,106]. Kusuma et al. derived early vascular cells from hiPSCs that were capable of maturing into both ECs and PVCs, organizing into vascular networks in hyaluronic acid based hydrogels[106]. The latter were infused with growth factors and implanted into immunodeficient mice. By day 3, vascular networks were demonstrable within the hydrogel. The hydrogels degraded in two weeks as designed. Explants were examined for a maximum duration of 2 weeks.

Our recent work demonstrated the longeveity of healthy hiPSC-engineered blood vessels in forming stable blood vessels *in vivo* (for 280 days in cranial window model, and 30 days in dorsal chambers in SCID mice)[37]. The EPCs were derived from hiPSCs using a novel triple combination of selection markers: CD34, neuropilin-1 (NP-1) and KDR, and an efficient 2-*D* culture system for hiPSC-EPC expansion. Non-invasive longitudinal *in vivo* microscopy was used to measure parameters, such as, permeability to bovine serum albumin**,** red blood cell (RBC) velocity and flow and to confirm function of blood vessels *in vivo* **(Figure 1, Videos 1 and 2).** The RBC velocities of engineered blood vessels were comparable to normal endogenous host vessels (1.36 ± 0.3 mm/s), and demonstrated a higher permeability as compared to endogenous vessels. For the first time it was also shown that durable hiPSC-derived blood vessels were formed from endothelial and mesenchymal precursor cell types that were isolated from the same hiPSC line.[37] Recently, another report validated that when human pluripotent stem cells were differentiated to ECs, the VE-Cadherin positive cells that were CD31+CD34+CD14–KDRhigh exhibited high angiogenic and clonogenic proliferation among ECs[143].

Clinical translation of hiPSC-derived vascular cells

Worldwide, there are currently 4,857 clinical trials registered at clinicaltrials.gov that involve

stem cells, with more than half of these in the USA (**Supplemental Figure S1**). Of these, 420 clinical trials use stem cells for the treatment of peripheral vascular disease, and 144 clinical trials utilize stem cells to treat diabetes. There are 21 clinical trials enlisted with Clinicaltrials.gov that have used or are using hESC-derived products, primarily for eye disorders such as macular degeneration. Of note, currently, there are 32 trials enlisted with clinicaltrials.gov involving hiPSCs, from predominantly the USA (n=24). [France (n=3), Israel (n=2), Spain (n=1), United Kingdom (n=1) and Slovenia (n=1).] These studies include characterization of hiPSC-derived cells from cardiovascular disease, neurological disease, retinal degenerative diseases and immunodeficiency disorders.

Currently there are no clinical trials that have utilized hiPSC-derived products for alleviating vascular disease such as peripheral limb ischemia or coronary heart disease. Thus, hiPSC-derived vascular cells have not yet impacted vascular tissue engineering. However, there is an increasing body of literature describing basic research using hiPSC-derived ECs and PVCs (*in vitro* studies and *in vivo* functional studies in mice), summarized in (**Supplemental Tables 2** and **3**).

The potential applications of hiPSC-derived vascular cells are wide, and include engineering blood vessels in coronary heart disease, alleviating critical limb ischemia in peripheral artery disease and examining the basis of diabetic vascular disease such as peripheral ischemia and potentially retinopathy. Heart disease and stroke remain outstanding causes of cardiovascular disease burden in the US. Coronary heart disease accounted for 1 in 6 deaths in 2009. The total burden of cardiovascular disease and stroke accounted for US $312.6 billion. This is startling when one considers that in 2008, the total cost of all benign and malignant neoplasms together in the USA was estimated at US $228 billion[144]. In 2010, stroke was the fourth leading cause of death in the USA. Worldwide, stroke is the second common cause of death after heart disease,

and is responsible for one-tenth of all deaths[145]. Thus, the potential benefit from the proposed approach is enormous.

Modeling vascular diseases using hiPSCs for diabetes

In 2011, more than 371 million people worldwide had diabetes, and more than 471 billion USD was spent on healthcare for diabetes. By 2030, 552 million are expected to have diabetes. 0.7% of these will develop retinopathy and become blind (http://www.idf.org/diabetesatlas/5e/Update2012). India is second to China in global prevalence of Type 2 diabetics (T2D), followed by the USA. Of the 63 million Indians with diabetes, it is alarming to note that a third of T2D have microvascular complications at presentation. In developing countries such as India, T2D vascular complications commonly result in diabetic foot ulcers and non-traumatic limb amputations[146]. hiPSC–derived vascular progenitor cells that could assist with revascularization of ischemic tissue could save limbs (**Figure 2**). However, in these vascular diseases, host endothelium is often dysfunctional[59,147,148] and it is possible that the hiPSC-derived cells from a diseased patient carry such memory. Conversely, the re-setting of the epigenetic landscape as a consequence of reprogramming may yield hiPSC-derived vascular progeny with differences from their parent cell, which has been demonstrated in mice[149].

Diabetic hiPSCs have been derived mainly for the differentiation or proposed differentiation to pancreatic β cells[67,150-154]. From a developmental perspective, however, it is known that the endothelium is instructive in providing signals that determine morphogenesis and cell differentiation of the pancreas[155]. The value of obtaining diabetic hiPSC derived vascular cells would be most robust in examining monogenic diabetes such as Type 1 Diabetes (T1D) or Maturity onset diabetes of the young (MODY) [156]. We have been successful in isolating hiPSC-

derived ECs and PVCs from Type 1 diabetes and MODY cases[37]. While the T1D-iPS engineered blood vessels were functional for 4 months, the MODY-iPS-ECs failed to form functional blood vessels *in vivo*, despite demonstrating bona fide endothelial tube forming potential *in vitro* in Matrigel[37]. Further studies are needed to confirm and build on these provocative findings.

T2D hiPSC lines from elderly patients are capable of generating insulin-producing beta cells[152]. In contrast, there is no published data to date demonstrating the derivation of vascular progenitor cells from diabetic hiPSCs. The derivation of T2D-hiPSC-ECs and T2D-hiPSC-perivascular cells might reveal a robust phenotype, which, even in the absence of a well-defined genetic background, may lead to mechanistic insights into vascular dysfunction in T2D. It is reasonable to speculate that our method of evaluation of hiPSC-engineered blood vessels from healthy controls, T1D and MODY patients[37] could be applied to investigate T2D-hiPSC vasculature.

Modeling non-diabetes vascular diseases using hiPSCs

hiPSC derived vascular cells have been investigated from rare vascular diseases with known genetic mutations (**Supplemental Table S3**). hiPSCs from the premature ageing disease, Hutchinson Gilford Progeria Syndrome were differentiated into 5 lineages: neural progenitor, endothelial, fibroblasts, vSMCs and MSCs[135]. The Hutchinson Gilford Progeria Syndrome-hiPSC-vSMCs survival was reduced under hypoxia, and Hutchinson Gilford Progeria Syndrome -hiPSC-MSCs were unable to prevent necrosis in a murine hind limb ischemia model, as compared with healthy MSCs. Williams Beuren Syndrome patient-derived fibroblasts from individuals with pulmonary and aortic stenosis were successfully differentiated to SMCs. These hiPSC-SMCs recapitulated the disease phenotype *in vitro* by demonstrating high proliferation and immature phenotype with low expression of differentiated SMC markers. The Williams

Beuren Syndrome-hiPSC-SMC phenotype was rescued by using rapamycin, a drug that inhibits smooth muscle proliferation. This result supports the testing of rapamycin in treatment of Williams Beuren Syndrome, and is an ideal example of the use of disease modeling with hiPSCs potentiating drug discovery or treatment modalities in the clinic. Supravalvular aortic stenosis–hiPSC-SMCs similarly displayed an abnormal SMC phenotype that was rescued by the addition of exogenous trophoelastin or expression of the Ras homolog family member A (Rho A)[114]. hiPSCs differentiated into cardiomyocytes from patients with LEOPARD syndrome demonstrated altered expression of various hypertrophy related genes and higher expression of TGF-β[136]. hiPSC-derived ECs from patients with Moyamoya disease, an idiopathic cerebrovascular disease that causes stroke in adults and children worldwide demonstrated impaired angiogenic ability, linked to the down regulation of the securin protein[137].

Besides using hiPSCs to model vascular disease, hiPSC-ECs have been useful in generating an *in vitro* blood brain barrier (BBB) model[86]. The BBB is composed of specialized ECs known as brain microvascular ECs, PVCs and astrocytes. The inaccessibility of these cellular types from humans has resulted in studies of the BBB from rodents, cows or pigs. A robust BBB of human origin was created for the first time by the use of hiPSC derivatives. When hiPSCs were co-differentiated into neural cell and EC lineage, these hPSC-ECs acquired BBB properties such as expression of tight junction proteins, glucose transporter, and P-glycoprotein. These hPSC-ECs demonstrate extremely high trans-endothelial electrical resistance, low molecular permeability and polarized transporter activity in response to astrocytes co-culture. This work is exciting as it offers a meaningful platform to examine developmental aspects of BBB formation and more clinically relevant issues such as opportunities to promote BBB repair after stroke or restrain the recruitment of blood vessels by aggressive brain tumors. The *in vitro*

BBB model may also permit assessment of delivery of drugs.

Conclusions and challenges in clinical translation of hiPSC-based engineered blood vessels

Only 7 years have passed since Shinya Yamanaka demonstrated in a landmark study that it was possible to reprogram adult somatic cells back to an embryonic stem cell-like state. This discovery led to the hope of hiPSC-derived cells could be used in the clinic to treat a myriad of diseases[81,157,158]. However, as discussed next, many challenges remain, especially in engineering 3-*D* tissues using hiPSCs.

Providing a vascular supply to newly generated tissue remains one of the most challenging barriers in building solid organs. Current situations of organ transplantation in the USA is dire: One patient is listed for transplant every 11 minutes, and 130 join this list every day. While 75 patients receive a transplant every day, 19 die due to non-availability of organs (http://www.thenationalnetworkoforgandonors.org/statistics.html). hiPSC derived vascular cells have broad application for regenerative medicine as blood vessels are essential for any functional organs as recently shown in the generation of functional liver in mice with co-implantation of hepatocytes, MSCs and vascular ECs[83]. The expectation is that genetically matched organs created will reduce the need for organ transplants in the future. But first we need to deal with such challenges as the variability of hiPSC lines and the long-term safety issues involved in the use of these cells, which are being addressed by researchers around the world. Animal models using hiPSC-derived retinal pigment epithelial cells showed negligible tumorigenic potential[159]. However, this issue remains important for current and future hiPSC clinical trial testing. We also need better ways of engineering organ-specific ECs. ECs are heterogeneous in both cellular content (arterial, venular or lymphatic) and function depending on anatomical location (fenestrated vs. non-fenestrated)[57]. Therefore engineering specific type of ECs or organ specific

ECs is biologically relevant and important for clinical application.

There are several outstanding issues that need to be addressed before hiPSC derived vascular progenitor cells find application in the clinic. Finding the appropriate vasculogenic clones and understanding the molecular mechanisms that confer vasculogenic potential are clearly more important than scaling up large number of hiPSC-vascular progeny. For example, we have shown that the selection of triple positive CD34+KDR+NP1+ cells provides highly vasculogenic cells, which form functional blood vessels *in vivo* when co-implanted with perivascular cells (**Figure 1**)[37]. We should also consider variability of hiPSC lines such as the cell of origin and reprogramming techniques. These factors will influence the success of hiPSC-vascular derivatives in vascular regenerative medicine. Would the timing of vascular insult be critical in vascular therapy using hiPSC-derived vascular cells – similar to spinal cord injury where immediate cell therapy is necessary in order to elicit a response? When translating mice to human data, one can imagine efforts to scale up hiPSC-derived vascular cells may require bioreactors, facilities for clinical grade production, biocompatible scaffolds and release devices to track the immune response *in vivo*. Another issue is the ability to obtain homogenous populations of cells derived from hiPSCs. Cost-effective therapy using hiPSC-derivatives remains a critical issue as manufacturing costs can be often prohibitive. The possibility that hiPSC derivatives might 'drift' to an immature or cancerous phenotype *in vivo* also remains a cause for concern[81,159]. Recent reports of tumors detected post treatment with stem cells reinforces the need for vigilant and prolonged follow-up[160].

Above all, in the rush to produce safe and biologically relevant hiPSC-derived vascular progeny, the dire reality is that host endothelium is often dysfunctional in vascular disease and it is possible the hiPSC-derived cells carry such memory. The mechanistic understanding of

healthy EC generation would help to overcome this problem. As of today, the only clinical trial in progress uses retinal pigment epithelium to treat age-related macular degeneration. Other proposed clinical trials involving hiPSC derivatives include platelets, genetically corrected keratinocytes to treat epidermolysis bullosa. Other possible trials using hiPSCs include oligodendrocyte precursor cells, dopaminergic neurons, retinal ganglion cells, keratinocyte and hematopoietic grafts[80,81]. The hope is that hiPSC-derived vascular progenitor cells too will find place in clinical trials to alleviate vascular insufficiency. Until this happens, hiPSC-derived vascular cells serve as an exceptional source to examine diseased cells in a dish (**Figure 2**).

Conflict of Interest: None

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FIGURE LEGENDS:

Figure 1: Multiphoton laser scanning microscopy image of engineered functional blood vessels *in vivo* in cranial window of SCID mice. hiPSC derived endothelial cells (green) co-implanted with perivascular 10 T ½ cells (red). Red blood cells labeled with DiD far-red dye (blue). [Reproduced from Samuel, et al. Generation of functionally competent and durable engineered blood vessels from human induced pluripotent stem cells. Proc Natl Acad Sci U S A 2013;110:12774-9.]

Figure 2: Potential application of hiPSC-derived blood vessels: Disease models and regenerative medicine.

Video 1: Multiphoton laser scanning image of iPS engineered blood vessels *in vivo* in Day 12 cranial window of SCID mouse: Green e-GFP HS27-iPS Endothelial cells co-implanted with DsRed 10 T 1/2 cells. The endothelial cells have formed well developed conduits forming arborizing networks of varying caliber that are supported by the perivascular 10 T ½ cells and embedded in the collagen 1 gel (Second harmonic generation signal, blue). Custom made MPLSM using confocal laser- scanning microscope body (Olympus 300; Optical Analysis Corp) and a broadband femtosecond laser source (High performance MaiTai, Spectra-Physics, Mountain View, CA). 20X magnification, 0.95NA water immersion objective was used (Olympus XLUMPlanF1, 1-UB965, Optical Analysis). Two-μm thick optical sections were taken. Imaging field of view was 660 μm x 660 μm x 155 μm with a resolution of 1.3 μm x 1.3 μm x 2 μm, 512 X 512 pixels with total of 228 slices taken.

Video 2: Multiphoton laser scanning image of iPS engineered blood vessels *in vivo* in Day 12 cranial window of SCID mouse: Green e-GFP HS27-iPS Endothelial cells co-implanted with DsRed 10 T 1/2 cells. Far-red labeled 1,1-dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine perchlorate (DiD) –red blood cells (RBCs) from species-specific mice (SCID) have been transfused to the SCID mouse via tail vein injection (blue) demonstrate RBCs traversing perfused blood vessels including anastomoses of host blood vessels with iPS-engineered vessel network. Custom made MPLSM using confocal laser- scanning microscope body (Olympus 300; Optical Analysis Corp) and a broadband femtosecond laser source (High performance MaiTai, Spectra-Physics, Mountain View, CA). 20X magnification, 0.95NA water immersion objective was used (Olympus XLUMPlanF1, 1-UB965, Optical Analysis).

Two-μm thick optical sections were taken. Imaging field of view was 660 μm x 660 μm x 155 μm with a resolution of 1.3 μm x 1.3 μm x 2 μm, 512 X 512 pixels with total of 228 slices taken. *(Video 2 corresponds to same region of interest as video 1).*

Supplemental Table 1: Key developments in functional evaluation of Human induced pluripotent stem cell derived/ Directed differentiation to endothelial cells (hiPS-ECs) and perivascular cells *in vivo*

Starting material/ cell lines	Reprogramming method	3D/ 2D	Reference
Adult dermal fibroblasts	Retroviral: OSKM	2D	1,2,3,4,
Adult dermal fibroblasts,	Retroviral: OSKM	3D	5,6,7,1
Adult dermal fibroblasts,	Retroviral: OSKM	Not known	8
Adult dermal fibroblasts,	Lentiviral: OSKM+ Nanog	2D	1
Adult dermal fibroblasts,	Lentiviral: OSK+ Nanog and Lin28	3D	9
Adult dermal fibroblasts,	Lentiviral: OS+ Nanog and Lin28	3D	10
Adult dermal fibroblasts	Plasmids: OSKM	2D	1
Human dental pulp	Retroviral: OS and OSKM	3D	11
Human lipoaspirate	Genome editing with zinc finger nuclease technology	3D	12
Human fetal/neonatal fibroblasts	Retroviral: OSK	2D	13
Human fetal/neonatal fibroblasts	Plasmid: OSKM+ Lin28	2D	14
Human fetal/neonatal fibroblasts	Lentiviral: OS + Nanog and Lin 28	2D	15
Human fetal/neonatal fibroblasts	Lentiviral: OSKM+ Lin28	2D	
Human fetal/neonatal fibroblasts	Lentiviral: OK	2D	16
Human fetal/neonatal fibroblasts	Lentiviral: OS+ Nanog, Lin 28	3D	17
Bone marrow	Lentiviral: OS, Nanog, Lin 28	3D	17
Bone marrow	Plasmid: OSKM + lin 28	2D	14
Type 1 Diabetic	Retroviral: OSKM	2D	1
Type 1 Diabetic	Retroviral: OSK	2D	1
Human foreskin fibroblasts and human hair keratinocytes	Retroviral: OSKM	3D	18
Human neonatal lung fibroblasts, Partially reprogrammed cells "PIPs"	Plasmid: OSKM	2D	19
Amniotic fluid	Lentiviral: OS + Nanog and Lin28	2D	20
Cord Blood	Plasmid: OSKM	3D	14

KEY: *OSKM: OCT4, SOX2, KLF-4, C-MYC*

3D refers to generation of three-dimensional embryoid body formation. 2D refers to two-dimensional co-culture conditions. N/A: not available.

Supplemental Table 2: Generation of Human induced pluripotent stem cell derived/ Directed differentiation to endothelial cells (hiPS-ECs) and perivascular cells.

Starting material/ cell lines	Reprogramming method / Feeders	3D/ 2D	Salient features	Ref.
Fetal and newborn foreskin fibroblasts and adult skin fibroblasts/	1. Fetal and newborn foreskin fibroblasts: Lentiviral: OS + Nanog 2. Adult skin fibroblasts: Lentiviral: OS + Nanog or OS + Nanog and Lin 28	2D	Differentiation pattern of hiPSC-ECs were similar to hESC	21
Adult dermal fibroblasts/	Retroviral: OSKM and OSK	2D	First report of both hiPSC derived endothelial cells and mural cells with efficiency comparable to hESCs	22
Fetal and newborn foreskin fibroblasts/	1. Lentiviral: OS+ Nanog and Lin 28 2. Retroviral: OSKM	3D	More than 50% of hiPSC-ECs showed beta galactosidase staining. Less than 5% of hESC-ECs exhibited senescence with positive staining for beta-galactosidase.	23
Adult dermal fibroblasts	Retroviral: OSKM	2D	Endothelial cells derived from hESCs and hiPSCs are comparable in function and proliferation and appear to be 'younger' than human aortic endothelial cells.	24
Fetal and newborn foreskin fibroblasts	Lentiviral: OS+ Nanog and Lin 28	3D	iPSCs derived from human dermal fibroblasts are similar to hESCs in capacity to generate endothelial (and hematopoietic) cells *in vitro*	25
Human dental pulp cells and skin fibroblasts	Retroviral: OS and OSKM	3D	Therapeutic response in hind limb ischemia and myocardial infarction mouse models within a week.	11
Adult dermal fibroblasts	Retroviral: OSKM	3D	Arterial, venous and lymphatic endothelial cells generated ECs	6
HUVECs	Retroviral: OSKM	2D	Endo-iPS cells similar to hESCs in morphology, gene expression, differentiation capacity and methylation pattern.	26
Fetal foreskin fibroblasts	Lentiviral: OS+ Nanog and Lin 28	3D	*In vitro* subculture of hiPSC-ECs showed limited growth rate as compared with hES-ECs.	27
Adult dermal fibroblasts	Retroviral: OSKM	3D	Overall findings suggest that early senescence is not an inevitable fate of hiPSC -ECs	28
Human amniotic fluid	Retroviral; OSKM	2D	Clonally expanded cardiovascular progenitor cells are capable of differentiating into cardiovascular, endothelial and smooth muscle lineage.	29
Dermal fibroblasts; neonatal and foreskin, and cord blood.	I. Integrative approach: Cord blood: Retroviral: OS Keratinocytes: Retroviral:OSKM or OSK and miR302-367. II. Non –integrative approach: 6-factor combination Oct-4, Sox-2, Klf-4, non-transforming l-myc (MYC1), Lin-28 and short hairpin RNA against p53		Partial de-differentiation reprogramming strategy led to multipotential progenitor cells from CD34+ population, including endothelial and smooth muscle cells. Endothelial cells arterial, venous and lymphatic phenotype.	30
Neonatal foreskin fibroblasts, BJ1 cell line Normal control (HFF) ,BMC1 Human dermal fibroblasts Human MSC line, MSC-1	Retroviral: OSKM Lentiviral: OSKM Modified –RNAs: OSKM Retroviral: OSKM+ hTERT, SV40 Large T ag	3D	ECs derived from hiPSCs were able to respond to biomechanical cues thereby directing differentiation into cells of the circulatory system. The hiPSC-ECs mounted an activated phenotype to pro-inflammatory stimuli, and revealed anticoagulant properties.	31

IMR90-TZ1 Bone marrowBM CD34 derived iPS cell line (BC1)	Lentiviral: OS+ Nanog and Lin28 1 or 2 EBNA/OriP plasmids used.	3D	Inhibition of TGF-β1 after mesoderm induction boosts cells of endothelial and hematopoietic lineage.	32
Fetal lung fibroblasts Bone marrow GFP transgenic hiPSC line from Human Cord blood derived endothelial cells	Retroviral: OSK Plasmid: OSKM + lin 28. Lentiviral: OS+ Nanog and Lin 28	2D	By Day 3, 'Early vascular cells' derived from hiPSCs were capable of maturing into both endothelial cells and pericytes.	14
Human Bone marrow derived mesenchymal stem cells and skin fibroblasts	Retroviral: OSKM	3D	Differentiated hBMMSC-iPSCs contained ` 20% CD34+ progenitor cells	33
Adherent cultures from 30 human samples, brain: cerebral cortical tissue and deep seated non-traumatic malignant lesions	Retrovirus mediated co-expression of transcription factors: Sox2 and Mash1. Reprogrammed cells termed human pericyte derived induced neuronal cells (hPdiNs)	2D	Cultures from adult human cerebral cortex with pericytic phenotype were reprogrammed to neuronal cells using the two transcription factors Sox2 and Mash1.	34
Human foreskin fibroblast and human hair follicle MSC	Lentiviral: OS +,Nanog, and Lin28 Lentiviral: OSKM	2D	A stepwise approach was taken to generate functional SMC through an intermediate highly proliferative MSC progenitor cell population.	35
Adult human dermal fibroblasts from excess skin of patient undergoing plastic surgery	Retroviral: OSKM	2D	SMCs derived from three progenitor subtypes (lateral plate mesoderm, paraxial mesoderm and neuroectoderm) displayed contractile activity and surrounded vascular structures in vivo.	36
Neonatal foreskin fibroblasts BJ-iPSC	Retroviral: OSKM	3D	TGF-β1 efficiently induced the differentiation of iPSC-NCSCs into SMC lineage.	37
Human iPSC line, SES 2, 5, 8, 9 and 10.	Lentiviral: OSK + Nanog and Lin28.	3D	iPSCs from human aortic vascular smooth muscle (HASMC) were redifferentiated back to SMCs.	38
Human fibroblasts	mRNA reprogramming: OSK	2D	iPSC-MSCs were functionally compatible with two 3D scaffolds tested, forming typically calcified structures within the scaffolds.	39
Bone marrow	Plasmid: OSKM	3D	MSCs were genetically modified for BMP2 delivery.	8
Adult fibroblasts	Lentiviral: OS+ Nanog and Lin28	3D	The small molecule SB431542 (TGF-β inhibitor) was used as a single-step method to direct the mesengenic differentiation of hiPSCs.	40
Amniotic fluid cells	Lentiviral:OS+ Nanog and Lin 28	2D	hiPS-MSCs inhibited Natural killer (NK) cell proliferation and cytolytic mechanisms.	20, 41
Foreskin	Retroviral: OSKM	N/A	MSC-like cells were derived from hiPSCs, in one step.	42
Foreskin	Lentiviral: OS+ Nanog and Lin28	2D	Bone marrow MSCs (BM-MSCs) display diminished proliferative capacity with repeat passages. The authors postulated that differential expression of ion channels in IPS-MSCs re responsible for their higher proliferative capacity than BM-MScs. It was discovered that the hEAG1 channel played a crucial role in the proliferative capacity of human IPS-MSCs and to a lesser degree, in BM-MSCs.	41

KEY: *OSKM: OCT4, SOX2, KLF-4, C-MYC*

3D refers to generation of three-dimensional embryoid body formation. 2D refers to two-dimensional co-culture conditions. N/A: not available.

Supplemental Table 3: Modeling diseases using human induced pluripotent stem cell derived endothelial cells (hiPS-ECs) / perivascular cells

Starting material/ cell lines	Reprogramming method	3D/ 2D	Salient features	Ref.
Hutchinson Gilford Progeria Syndrome (HGPS): HGPS patient fibroblast cells from Coriell cell repositories	Retroviral: OSKM	3D	HGPS-IPS cells differentiated into neural progenitor cells, endothelial cells, fibroblasts, VSMCs and MSCs. HSPG-iPS-MSCs were unable to prevent necrosis in a hind limb ischemia model, in comparison with healthy MSCs.	43
Fetal foreskin fibroblasts Human foreskin fibroblasts	Lentiviral: OS+ Nanog and Lin28 Episomal vectors: OSKM+ Nanog, Lin-28, SV40LT and hTERT .	2D	hIPS-ECs acquire BBB properties when co-cultured with neural cells..	44
Williams Beuren syndrome (WBS) fibroblasts from patient with pulmonary and aortic stenosis was used. Human aortic vascular smooth muscle and HUVECs were controls.	Retroviral: OSKM.	3D	The WBS-iPS-SMCs recapitulated the disease phenotype *in vitro.* The abnormal WBS-iPS-SMC phenotype was rescued by up regulating the ELN signaling pathway, or by using rapamycin.	45
Vascular SMCs derived by explant outgrowth from excised epicardial coronary artery of Supravalvular aortic stenosis (SVAS) patient	Lentiviral; hSTEMCAA polycistronic vector:OSKM	3D	SVAS-iPS-SMCs produced reduced amounts of elastin, reduced actin polymerization, increased proliferation and increased migration as compared to wild type controls. The abnormality was rescued by the addition of exogenous trophoelastin or Rho A.	46
Adult dermal fibroblasts from patients with LEOPARD syndrome and human dermal fibroblasts	Retroviral: OSKM	3D, and 2D	Selection of CD166 provided a pure population of CMs (>90%). Techniques utilized isolated non-CMs also (ECs and SMCs). L2 iPS6-CM showed altered expression of various hypertrophy related genes and higher TGF-beta. Hypertrophic cardiomyopathy is a major disease type in patients with LEOPARD syndrome.	47
Dermal fibroblasts from 3 patients with Moyamoya Disease (MMD); unaffected carrier, and two healthy controls taken	Retroviral: OSKM	2D	Angiogenic activities of iPS-ECs from MMD patients and carrier were lower than wild type cases, with down regulation of the gene Securin.	48

KEY: *OSKM: OCT4, SOX2, KLF-4, C-MYC*

3D refers to generation of three-dimensional embryoid body formation. 2D refers to two-dimensional co-culture conditions. N/A: not available.

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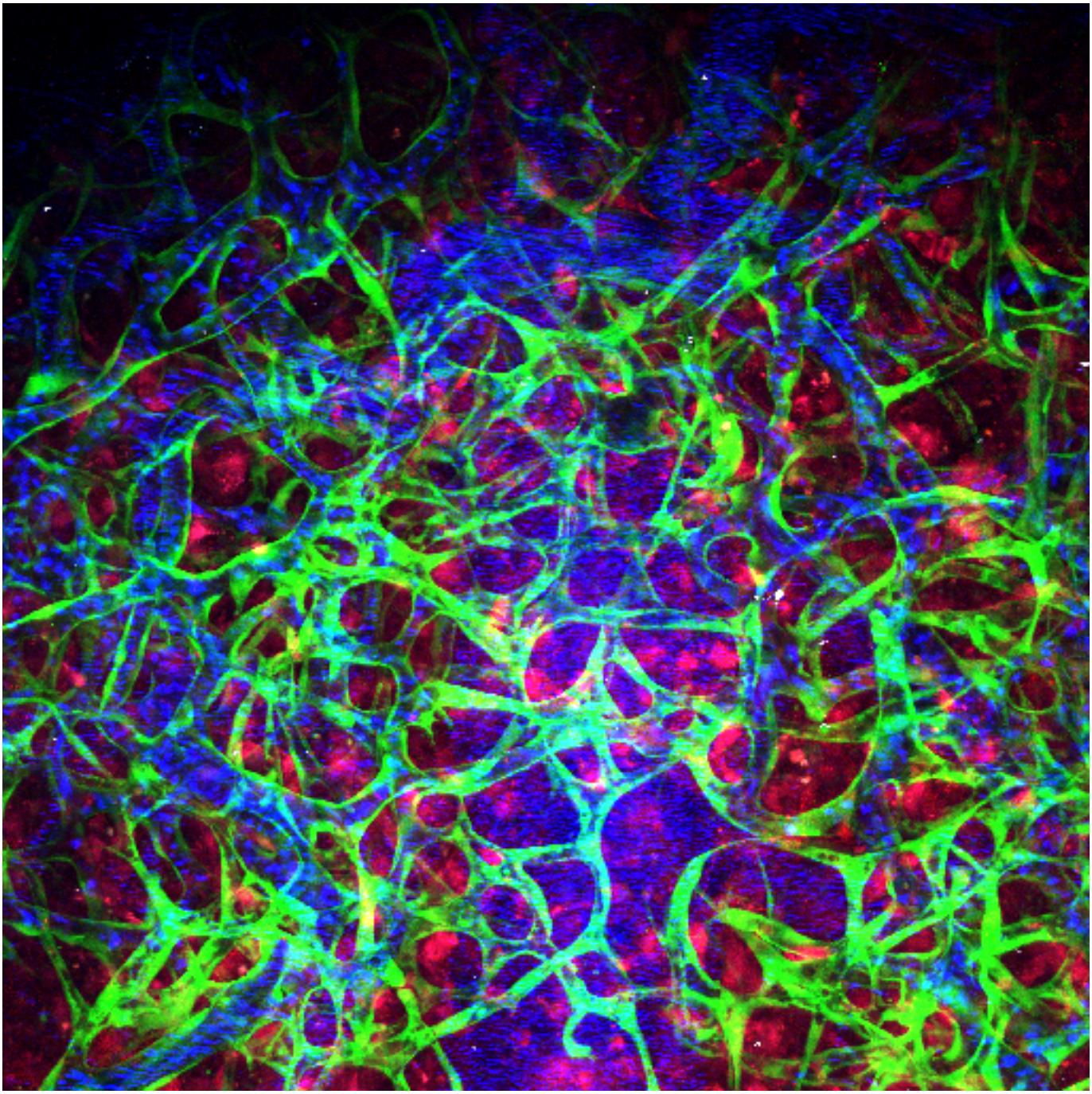


Figure 1: Multiphoton laser scanning microscopy image of engineered functional blood vessels in vivo in cranial window of SCID mice

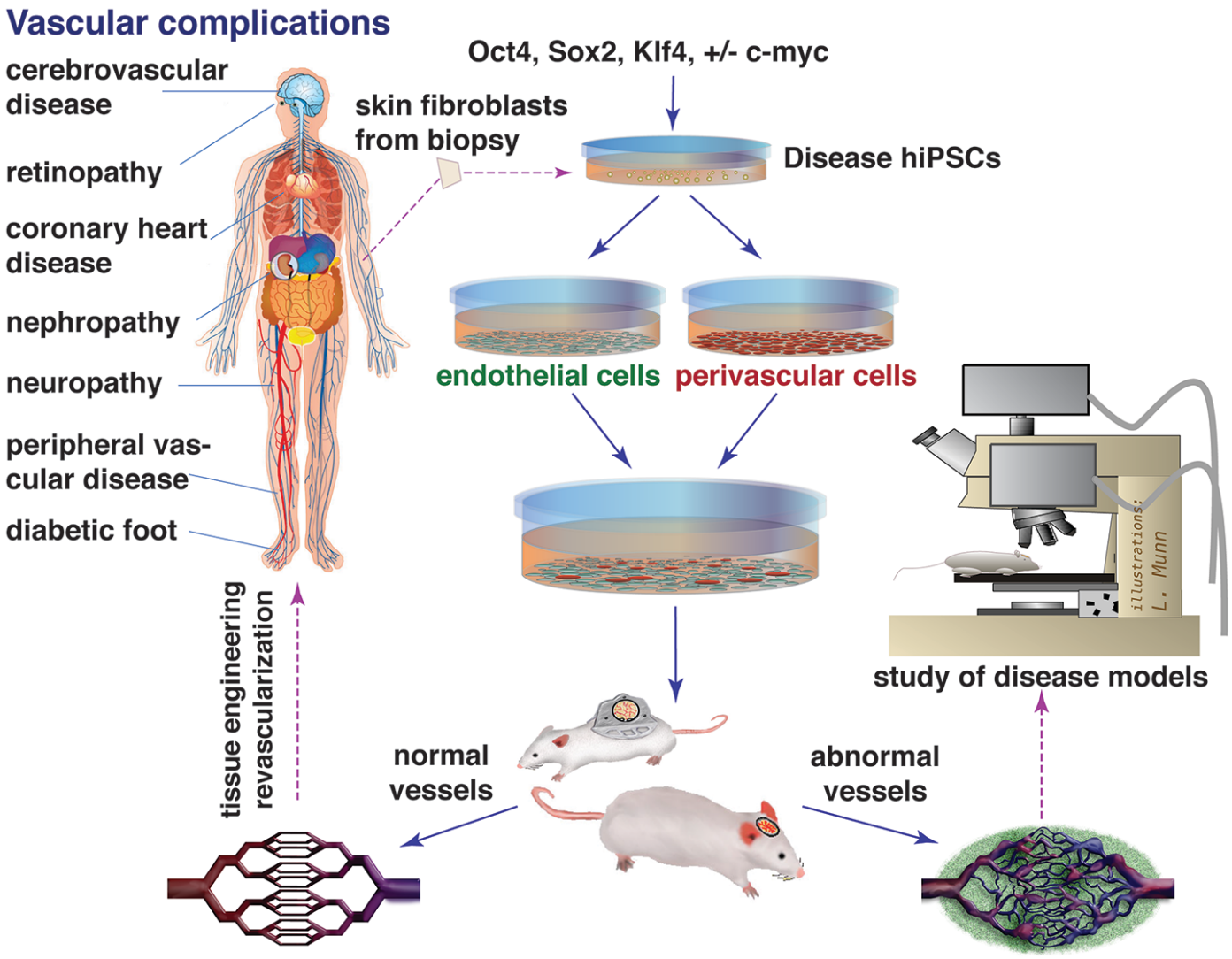
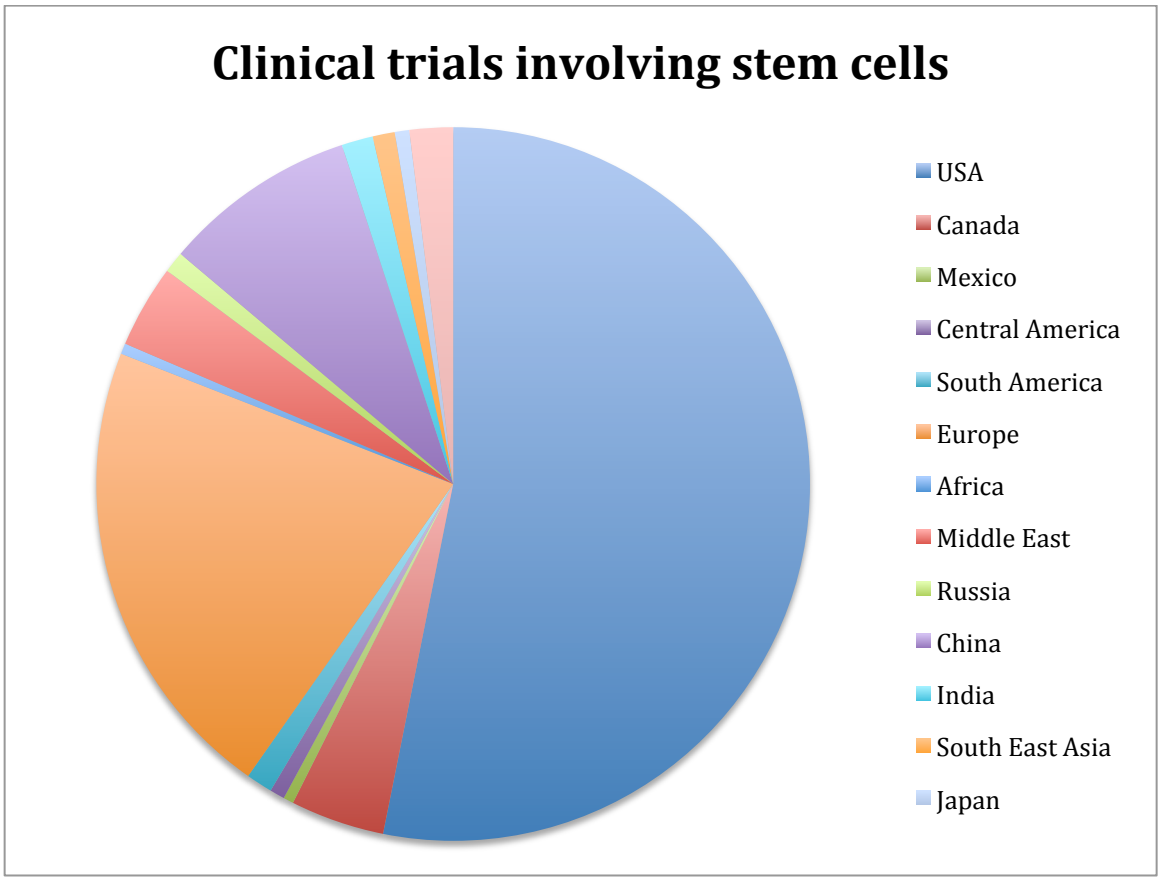


Figure 2: Potential application of hiPSC-derived blood vessels: disease models and regenerative medicine.

Supplemental Figure 1



Reference: *clinicaltrials.gov*